PHYSICAL AND BARRIER PROPERTIES OF DEVELOPED BILAYER PROTEIN FILMS

J. A. Foulk, J. M. Bunn

ABSTRACT. *The effects of three bilayer–forming methods (liquid protein spread on dry film, heat pressing dry films, and solvent laminating dry films) on physical and barrier properties were investigated. These bilayer films were generated using soy protein isolate, wheat gluten protein, and corn zein protein. Bilayer films were evaluated for enhanced properties (tensile strength, percent elongation, water vapor, and oxygen permeability) over single layer films. Using X–ray crystallography, these films showed no changes in degree of crystallinity. Depending upon film type in this study, protein bilayer films exhibited reduced, similar, and enhanced properties. Hydration properties, solvent and protein interactions, film thickness, surface properties, or heat and pH differences influenced the formation of these films. Water vapor permeability for these films ranged from 1.3×10^{-9} g m^{-1} s^{-1} Pa^{-1} for soy sprayed on corn film to 3.5×10^{-9} g m^{-1} s^{-1} Pa^{-1} for solvent laminating wheat and soy films. Oxygen permeability ranged from 2.67×10^{-17} moles m $m^{-2}s^{-1}$ Pa^{-1} for spraying soy on corn films to 8.81×10^{-19} moles m $m^{-2}s^{-1}$ Pa^{-1} for pouring soy on wheat film. Tensile strength ranged from 1.57 MPa for spraying corn on wheat films to 9.10 MPa for heat pressing wheat and corn films. Elongation ranged from 5.36% for pouring soy on corn films to 204.12% for pouring soy on wheat films. Bilayer films appear to exhibit reduced tensile strength, similar oxygen permeability, enhanced percent elongation, and greatly increased water vapor permeability.*

Keywords. *Edible films, Bilayer films, Biopolymer films, Tensile strength, Permeability, Protein films.*

In the last several years, considerable research has centered on the idea of using edible protein films and other biopolymers to produce a more environmentally friendly film. Presently edible films do have limitations on their water vapor permeability and tensile strength compared to synthetic polymer films.

Multilayer films of different protein materials may have improved properties over a single layer film. Monolayer films used in producing bilayer films have different properties and thus various advantages. Corn zein films have lower water vapor permeability and higher tensile strengths. Soy protein isolate and wheat gluten films have lower oxygen permeability and a higher percentage elongation.

Bilayer films of the past have simply been formed by applying the second film layer on top of the first using one of the following methods: thin layer chromatography, painting, spraying, dipping, or an emulsion technique. All of these previous films were constructed with a structural layer for mechanical support and a lipid layer to enhance barrier properties. Little is known about processes for producing pure protein bilayer films. But, production methods may influence bilayer film characteristics and could be a determining factor in developing a usable multilayer protein film.

Methods must be developed to produce these films, evaluate their properties and compare them with single layer films. To establish a method to produce bilayer films from protein materials, techniques used for lipid bilayer films construction were consulted and bilayer films were formed from the protein fractions of corn, soybean, and wheat by three different techniques.

OBJECTIVES

The objectives of this study were to:

a) Produce bilayer protein films by three different preparation methods (liquid protein spread on dry film, heat pressing dry films, and solvent laminating dry films),
b) Compare barrier and physical properties of bilayer films to single layer films from the same base materials.

LITERATURE REVIEW

According to Gennadios and Weller (1990), biopolymer films and coatings can be defined as thin layers of edible material applied on (or within) foods by wrapping, immersing, brushing, or spraying in order to offer a selective barrier against the transmission of gases, vapors, and solutes while offering mechanical protection. Over the last several years, research has indicated that biopolymer films can act as moisture, gas, and solute barriers (Kester and Fennema, 1986; Gennadios and Weller, 1990, 1991; Park et al., 1992; Brandenburg et al., 1993; McHugh et al., 1993; Foulk, 1994; Park et al. 1994; Gennadios et al., 1994).

Article was submitted for review on October 1999; approved for publication by the Food & Process Engineering Institute of ASAE in March 2001. Presented at the 1994 ASAE International Summer Meeting as Paper No. 94–6017.

Technical Contribution No. 4466 of the South Carolina Agriculture and Forestry Research System, Clemson University.

The authors are **Jonn A. Foulk**, Research Associate, Cotton Quality Research Station, USDA–ARS, Clemson, South Carolina, and **Joe M. Bunn**, ***ASAE Fellow Engineer***, Emeritus Professor and Chair, Department of Agricultural and Biological Engineering, Clemson University, South Carolina. **Corresponding author:** J. A. Foulk, USDA–ARS CQRS, Clemson University, Ravenel Center, RM. 10 McGregor Road, Clemson, SC 29634; phone: 864–656–2488; fax: 864–656–1311; e–mail: Jonnf@CLEMSON.EDU.

  ISSN 0883–8542

Biopolymer films, derived from biological substances, are usually categorized into three categories: polysaccharide, lipid, and protein films (Kester and Fennema, 1986). A non–exhaustive list of edible substances that are used in production of these films includes wheat gluten proteins, soy protein isolates, corn zein proteins, starches, pectin, various lipid compounds, meat proteins, cellulose derivatives, casein, chitosan, collagen, and keratin. These products can be used alone or in combination to form a coating, which is applied directly to the product; a film, which is used to cover a product; multilayer film or coating; or an emulsion.

Many multilayer films have been developed that combine two or more materials containing properties which offset one another (Kamper and Fennema, 1984a, 1984b; Kester and Fennema, 1986, 1989a, 1989b; Vojdani and Torres, 1989, 1990; Hagenmaier and Shaw, 1990; Park and Chinnan, 1990; Rico–Pena and Torres, 1991). Since lipids provide a superior water vapor barrier, which few other films can offer, they have been used for production of many bilayer films. These films provide low values for water vapor and oxygen permeability, but are very sensitive to fractures caused by stress. Bilayer films would be candidates for the same commercial markets as single layer films, which have been addressed by Narayan (1994).

Protein films formed from wheat gluten have good film forming capabilities due to a unique cohesive and elastic property of the gluten (Wall and Beckwith, 1969). This protein derived from wheat is considered hydrophilic. Gliadins are the low molecular weight portion of wheat gluten while glutenins contain higher molecular weights (Cheftel et al., 1985). Wheat gluten is a protein network held in place with covalent and noncovalent bonds. Smaller globular gliadin polypeptides are packed into the network and interact with the glutenins. Generally, gliadins bond noncovalently with the glutenins (Pomeranz, 1988).

According to Freeman Industries Inc. (1986), corn zein has an amino acid composition and solubility similar to wheat gliadin. Zein is insoluble in water because it contains the following amino acids, which are hydrophobic: leucine 15.4%, proline 10.0%, and alanine 10.5%. It is also hydrophobic because it contains a high proportion of hydrocarbon group side chains, and amide groups with few free carboxylic acid groups (Freeman Industries Inc., 1986). Corn zein has been found to be a strong antioxidant that can be beneficial in producing a packaging film (Wang et al., 1991).

Soybean proteins consist mainly of globulins. Soy protein isolate is a refined form of soybean protein in which the water insoluble polysaccharides, and other low molecular weight components, have been largely removed (Wolf, 1970). Soy protein isolate is hydrophilic. This protein has poor antioxidant properties compared to corn zein and wheat gluten (Wang et al., 1991).

Heating during the mixing of soy films is necessary because the bonds are denatured by the additional heat which changes the three–dimensional structure of proteins exposing sulfydryl groups and hydrophobic side chains (Gennadios and Weller, 1991). Soy protein films are formed from a combination of disulfide, hydrophobic, and hydrogen bonds. Partially unfolded molecules exhibit some hydrophobic properties (Kinsella, 1979). As the films dry, these groups approach each other forming disulfide and hydrophobic bonds. These hydrophobic ends are exposed on the film face while the hydrophilic groups are positioned toward the glass plate on which a film is dried (Okamoto, 1978).

Materials and Methods

Wheat gluten, corn zein, and soy protein isolate were the three protein materials chosen for producing bilayer films. Corn zein and wheat gluten films were prepared according to Park et al. (1994) and soy protein isolate films were prepared according to Brandenburg et al. (1993). Controlling film–forming properties is critical to the development of proper barrier and physical properties. Therefore, forming techniques were practiced and films evaluated until all three types of single layer films were produced with measured tensile strengths within a 95% confidence interval of these researchers' published data.

Bilayer films were formed by three techniques. The first method involved forming one protein film and after it dried forming another layer, by pouring, spraying, or rolling, directly on top of the previously dried layer. This combined film was then allowed to dry according to procedures followed for single layer film. Pouring the second layer onto the first layer was done in a manner described by Brandenburg et al. (1993) or Park et al. (1994) for single layer films. Spraying a second layer was accomplished with a worm screw that allowed an electric paint sprayer to move at a constant speed while dispensing a uniform amount of solution. Spraying was used to coat the first layer with a very thin second layer. A thin layer would dry quicker so that the dried film was not in contact with the dissimilar liquid solution for any extended time period. The sprayer could only be used to spray corn zein and soy protein isolate solution; that is, wheat gluten could not be sprayed. Rolling a second layer could only be done with the corn zein because this technique produced many air bubbles in soy protein isolate and wheat gluten. In the roller technique a 7.6–cm foam roller produced by SHUR–LINE® was used. This foam roller had 20 pores/cm.

The second method involved forming two monolayer films individually and then laminating these together using a solvent between them while applying a uniform pressure. This second method involved producing two single layer films separately on one day. The following day these films were coated, on the side opposite the surface in contact with the drying glass during single film formation, with 95% ethanol alcohol using a SHUR–LINE® foam roller. The alcohol–covered sides of the films were placed together and any visible air bubbles existing between the layers were carefully pressed out. These two layered films were positioned between sheets of silicone coated paper and this multilayer "sandwich" of paper and film passed through compression rolls in a Laboratory Hot Melt, type 500 calendar rolls produced by Ernst Benz Ag, Rumlang–Zurich. These "sandwiches" were fed through the machine at a maintained speed of 3.4 m/min and a pressure of 981 KPa.

The newly formed bilayer film was removed from the silicone coated paper sandwich and placed between sheets of uncoated paper to await conditioning for properties testing. The silicon paper was made from pure Kraft pulp and coated on both sides with a silicone emulsion. All raw materials and additives used in production of this paper are approved for human consumption by FDA regulations. The paper is suitable for cooking, baking, and frying in both conventional and microwave ovens since the silicone is stable to high temperatures, oxidation, chemical, and biological environments (Odian, 1993).

A third production method involved forming two monolayer films individually laminating them together using heat and pressure from a set of rolls. This method involved producing two single layer films separately on one day. The following day one film was placed against the second film in a way similar to method two except no solvent was used. Any visible air bubbles were carefully pressed from the film. In a manner similar to method two, these combined films were placed between silicone–coated paper and this multilayer "sandwich" passed through the calendar rolls that in this case were heated. A feed speed of 3.4 m/min was maintained with calendar roll surface temperatures of 135, 141, and 146°C used for temperature treatments. These surface temperatures were measured using a hand held measuring instrument (Omegatemp model HH–1).

The calendar rolls were heated by circulating heated oil at a rate of 25 L/min that was controlled by a Haake two–point temperature controller. As a "sandwich" of film materials passed through the calendar rolls it was pressed together at a pressure of 294 KPa. The newly formed bilayer film was removed from the silicone–coated paper, placed between sheets of uncoated paper, and held for conditioning and testing.

The three techniques used in bilayer film formation did not always produce films. To help categorize these films, they were divided into the following three classes: favorable, irregular, and unsatisfactory combinations. Favorable combinations resulted in a smooth, uniform bilayer film surface (approximately 900 cm^2), while irregular (substandard) combinations resulted in a rough and uneven bilayer film surface with areas of usable film approximately 49 cm^2. Bilayer films with negligible and many fragmented testable areas were deemed unsatisfactory combinations due to few or no areas larger than 49 cm^2.

CONDITIONING AND TESTING

All conditioning and testing procedures were identical to those used by Park et al. (1994) and Brandenburg et al. (1993). Bilayer films for physical properties and water vapor barrier properties testing were conditioned for 48 h immediately before testing in an electronically controlled environmental chamber. The model FRS–251C–1 (G. S. Blue M Electric) was maintained at 25 ± 2°C and 50 ± 3% relative humidity. The chamber had a horizontal airflow velocity of 54 m/min. Film for oxygen testing was conditioned in a desiccator at 23 ± 2°C and 0 ± 5% relative humidity. Relative humidity was maintained within the desiccator using Drierite.

FILMS THICKNESS

Test film thickness was determined using a hand held micrometer (B.C. Ames Co.). The micrometer had 6–mm diameter flat measuring faces. For each film type and test, five separate measurements were taken to the nearest 0.0254 mm, systematically over the cross section of each test sample.

FILMS PHYSICAL PROPERTIES

A film's peak load and extension was determined using a model 4201 Instron Universal Testing Machine, operated according to ASTM standard method D 882–88 (ASTM, 1989). Initial grip separation and crosshead speed were set at 50 mm and 500 mm/min, respectively. Tensile strength was determined at maximum load and percent elongation was determined at break. Unit tensile strength and percent elongation were calculated using methods described by Brandenburg et al. (1993) and Park et al. (1994).

Crystalline structure of biopolymer films was determined using a Scintag XDS2000 diffractometer with a lithium drifted silicon solid–state detector. The X–ray source was a copper X–ray using $K\alpha_1$ radiation. Samples were scanned at a rate of 3 degrees/min, which is recommended for qualitative work. All single layer and bilayer films were tested.

FILMS WATER VAPOR PERMEABILITY

Modified cups were used to determine water vapor permeability of films. These cups consisted of a lid and bottom section, which were fabricated from polymethylmethacrlate (Piedmont Plastics, Inc., Greenville, S.C.). The cups were made according to a design by Dr. J. M. Krochta, Department of Food Science and Technology, University of California, Davis, and were manufactured at Clemson University (Gennadios et al., 1994). Schematic and dimensions of the cup are outlined in Foulk (1994). The bottom part of a cup was filled with 18 mL of distilled water that allowed a 9.97–mm air gap to exist between a covering film and the water surface. The exposed film area, through which the water vapor transmission was to be measured, was 16.62 cm^2.

These cups were placed within an environmentally controlled chamber maintained at 25 ± 2°C and 50 ± 3% relative humidity. Permeability was measured following procedures outlined by Brandenburg et al. (1993) and Park et al. (1994). Not accounting for the stagnant air gap between the water surface and hydrophilic film can lead to an underestimation of film permeability therefore, calculations were performed to account for this air gap resistance which provided the corrected permeability values reported here. These correction calculations were made according to procedures presented by McHugh et al. (1993) and Gennadios et al. (1994).

FILMS OXYGEN PERMEABILITY

Oxygen permeability was determined using an OX–TRAN 1000 (Mocon Inc., Minneapolis, Minn.). This machine was operated according to ASTM Method D 3985–81 (ASTM, 1989). Conditioned films were placed in precut aluminum masks that allowed an exposed area of 5 cm^2. Testing was done at 25°C and 0% relative humidity. Films were placed in the OX–TRAN machine and flushed with nitrogen for 18 h before test measurements were obtained. Procedures were followed according to Brandenburg et al. (1993) and Park et al. (1994).

RESULTS AND DISCUSSION

Producing a bilayer film by topping a dry film with a liquid film produced acceptable films in most but not all combinations (tables 1, 2, and 3). Some combinations caused altering of the protein in the previously dried film. Incompatibilities among proteins, which likely influenced the formation of these bilayer films, could be related to hydration properties, solvent and protein interactions, film thickness, surface properties, or heat and pH differences.

Bilayer Film Formation

Pouring

Pouring wheat on corn caused the corn layer to whiten in random areas. Samples from these white areas were not tested because they exhibited poor flexibility. Pouring corn on wheat in turn altered the wheat in selected areas and samples from these areas were not tested.

Pouring soy on corn caused the entire plate of corn to whiten and become brittle to the point where it simply flaked off the glass and could not be recovered as film. Pouring corn on soy caused the combined film to have an oily feel because some plasticizer was exposed on the surface. Pouring corn on soy also had a few irregular areas that were not tested because of the appearance of the film as illustrated in Foulk (1994).

Pouring soy on wheat caused the dried wheat to pucker randomly across the glass plate as illustrated in Foulk (1994). Test samples were selectively removed so as not to include these areas in tested materials. These raised areas had increased thicknesses and were very brittle. Pouring wheat on soy encountered similar problems with puckering so those samples were selectively removed.

Spraying

Wheat could not be successfully sprayed using the spraying apparatus available because as the wheat solution cooled its viscosity increased dramatically. Spraying soy appeared to work well. Spraying corn on soy also caused combined film surfaces to have an oily feel due to excessive plasticizer at the surface.

Rolling

Neither soy nor wheat was successfully rolled since the foam roller always produced bubbles in the films. Films containing bubbles were considered defective because they were not uniform in nature. Corn was rolled onto wheat and soy without problems.

Intermediate Solvent

Films produced with an intermediate solvent had some wrinkles as if the “sandwich” of materials was not properly fed through the calendar rolls. No wrinkled films were tested. All film combinations were used to produce a bilayer film with this technique. Surfaces of these films had a look and texture similar to the silicon–coated paper used in their formation.

Heat

All combinations of single layer films were used in producing bilayer films with this technique. Some wrinkles were created as if the “sandwich” of materials were not fed correctly through the calendar rolls. Wrinkled films were not tested. Surfaces of these films also had a similar look and texture as the silicon coated paper used in their formation.

Water Vapor Permeability

Water vapor permeability was determined with different sides of the films facing the high humidity area and it was statistically shown that there was no significance difference between sides. Table 1 displays water vapor permeability for all techniques of bilayer protein films including single layer protein films produced by Brandenburg et al. (1993) and Park et al. (1994). Calculations were performed to provide corrected water vapor permeability values (see Gennadios et al., 1994, for calculation details). Water vapor permeability was a function of bilayer film production technique. Some observed differences between films are due to thickness differences. Kanig and Goodman (1962), Banker et al. (1966), and McHugh et al. (1993) have all shown that water vapor permeability increases as thickness increases. Water vapor permeability for the combination wheat/soy bilayer films were above the water vapor permeability values reported for either wheat or soy single layer films. This

Table 1. Water vapor and oxygen permeability values for single and bilayer films.[a]

Single Layer Films			Water Vapor Permeability[b] ($g\ m^{-1}\ s^{-1}\ Pa^{-1}$)	Oxygen Permeability[c] ($moles\ m\ m^{-2} s^{-1}\ Pa^{-1}$)
Wheat gluten[d]			1.84E–9	4.95E–18
Soy protein[e]			1.90E–9	1.34E–18
Corn zein[d]			0.80E–9	1.16E–17
Bilayer Films[f]				
Method	Type	Quality		
ss	cs	F	1.30E–9 i	2.67E–17 a
h9	cs	F	1.37E–9 h,i	1.57E–17 b
h8	cs	F	1.40E–9 h,i	3.71E–18 f,g,h
sc	cs	I	1.38E–9 h,i	2.58E–18 g,h
r	cs	F	1.49E–9 f,g,h,i	3.00E–18 f,g,h
ps	cs	U	1.57E–9 e,f,g,h,i	n.a.
a	cs	F	1.68E–9 e,f,g,h	4.95E–18 d,e,f,g
h7	cs	F	1.79E–9 d,e,f	5.60E–18 d,e,f,g
pc	cs	I	1.83E–9 d,e,f	4.29E–18 e,f,g,h
sc	wc	F	1.42E–9 g,h,i	5.57E–18 d,e,f,g
pw	wc	I	n.a.	1.20E–17 c
h8	wc	F	1.59E–9 e,f,g,h,i	6.86E–18 d,e,f
h9	wc	F	1.61E–9 e,f,g,h,i	8.09E–18 d,e
pc	wc	I	1.76E–9 d,e,f,g	5.75E–18 d,e,f,g
a	wc	F	1.81E–9 d,e,f	8.49E–18 d
h7	wc	F	1.90E–9 d,e	7.57E–18 d,e
r	wc	F	2.06E–9 d	7.53E–18 d,e
ps	ws	I	2.77E–9 c	8.81E–19 h
h9	ws	F	3.08E–9 b,c	2.96E–18 g,h
h8	ws	F	3.33E–9 a,b	3.12E–18 f,g,h
h7	ws	F	3.45E–9 a	8.56E–18 d
pw	ws	I	3.40E–9 a	3.16E–18 f,g,h
a	ws	F	3.50E–9 a	8.01E–18 d,e

[a] Values followed by different letters within columns are significantly different, $P < 0.05$, according to Duncan’s New Multiple Range Test.
[b] Water vapor permeability values were evaluated at 25°C and 50% RH.
[c] Oxygen permeability values were evaluated at 25°C and 0% RH.
[d] From Park (1993) and Park et al. (1994).
[e] From Brandenburg et al. (1993).
[f] Table 2 includes film thickness values and table 3 defines abbreviations.

increase in water vapor permeability values indicates that no tested method for wheat/soy bilayer protein films appeared to drastically enhance the properties beyond either single layer film. Certain combinations of corn/soy and wheat/corn bilayer films did appear to have enhanced properties beyond soy protein and wheat gluten single layer films. However, no corn/soy or wheat/corn bilayer combinations enhanced the water vapor permeability beyond the single layer corn zein films.

Oxygen Permeability

Table 1 presents oxygen permeability values for all techniques of bilayer protein films including single layer protein films produced by Brandenburg et al. (1993) and Park et al. (1994). Permeability was a function of the bilayer film production technique. Oxygen permeability values for the wheat/soy combination were scattered above and below oxygen permeability values for either wheat or soy single layer films. For these wheat/soy bilayer films, most oxygen permeability values appeared slightly improved over single layer wheat gluten films. However, only one wheat/soy bilayer combination enhanced the water vapor permeability beyond the single layer soy protein films. The bilayer film with soy poured over the wheat was the one combination that reduced oxygen permeability values below those for either wheat or soy. Typically, corn/soy and wheat/corn combination bilayer films displayed oxygen permeability values higher than the superior single layer film and lower than the inferior single layer film. Certain combinations of corn/soy and wheat/corn combination bilayer films did appear to have enhanced properties beyond corn zein and wheat gluten single layer films. However, no corn/soy or wheat/corn bilayer combinations appeared to enhance the oxygen permeability beyond the single layer soy protein film.

Thickness

Film thickness varied with production technique but with an indicated possibility of producing thinner films with further research. Table 2 shows the thicknesses for the different bilayer films studied. All films produced were thicker than any single layer protein film previously produced.

Tensile Strength

Table 2 compares tensile strengths of formed bilayer protein films to single layer protein films produced by Brandenburg et al. (1993) and Park et al. (1994). Tensile strength was a function of bilayer film production techniques. Film combinations wheat/soy, wheat/corn, and corn/soy produced several films with enhanced properties over wheat gluten and soy protein single layer films. However, no tested bilayer combinations appeared to enhance the tensile strength beyond the single layer corn zein film. The film combination wheat/soy produced tensile strengths above and below both wheat gluten and soy protein single layer films. When alcohol was used as a solvent between two film layers and wheat was poured on soy the bilayer films produced had higher tensile strengths than single layer wheat or soy films. Wheat/corn bilayer films displayed tensile strength values above and below single layer wheat gluten films but none higher than corn zein single layer films. Corn/soy combination films exhibited properties above and below single layer soy protein films but again none higher than corn zein single layer films. Not all film combinations had enhanced properties over single layer films, some tensile strengths were greatly reduced, for example pouring corn on soy.

Table 2. Thickness, tensile strength, and film elongation values for single and bilayer films.[a]

Single Layer Films				Thickness (mm)[b]	Tensile Strength (MPa)	Elongation (%)
Wheat gluten[c]				0.129	3.45	103.00
Soy protein[d]				0.068	4.33	78.18
Corn zein[c]				0.124	13.40	74.19
Bilayer Films[e]						
Method	Type	Quality	No.			
pc	cs	I	32	0.197	2.83 h	9.83 g
ss	cs	F	20	0.166	2.96 g,h	84.53 d
sc	cs	I	12	0.128	3.04 g,h	5.69 g
r	cs	F	40	0.125	4.61 f	15.42 g
h7	cs	F	50	0.233	5.54 e	158.34 b
ps	cs	U	8	0.185	6.04 d,e	5.36 g
a	cs	F	95	0.224	6.67 c	136.08 c
h8	cs	F	50	0.197	7.48 b	70.15 d,e
h9	cs	F	40	0.217	7.51 b	54.33 e,f
sc	wc	F	18	0.224	1.57 i	167.53 b
r	wc	F	103	0.172	2.08 i	192.79 a
pw	wc	I	24	0.332	2.95 g,h	87.06 d
pc	wc	I	28	0.204	3.33 g,h	119.96 c
a	wc	F	55	0.265	5.61 e	137.82 c
h8	wc	F	40	0.224	6.80 c	128.96 c
h7	wc	F	28	0.218	7.90 b	120.47 c
h9	wc	F	30	0.218	9.10 a	71.00 d,e
ps	ws	I	35	0.174	1.63 i	204.12 a
h7	ws	F	55	0.215	3.11 g,h	158.70 b
h8	ws	F	35	0.206	3.42 g,h	158.00 b
h9	ws	F	26	0.202	3.50 g	163.26 b
a	ws	F	64	0.226	6.42 c,d	49.29 f
pw	ws	I	19	0.262	4.83 f	127.64 c

[a] Values followed by different letters within columns are significantly different, $P < 0.05$, according to Duncan's New Multiple Range Test.
[b] Thickness values represent means of 5 measurements per film sample.
[c] From Park (1993) and Park et al. (1994).
[d] From Brandenburg et al. (1993).
[e] Table 3 defines abbreviations.

Film Elongation

Film elongation for bilayer and single layer films produced by Brandenburg et al. (1993) and Park et al. (1994) are compared in table 2. These values show that film elongation was greatly influenced by the method of production. The combination corn/soy had film elongation values that were generally less than either single layer corn or soy film. In all but one technique the combination wheat/soy had enhanced film elongation properties over both single layer wheat and soy films. Thickness is not accounted for in calculating elongation values and thickness differences between single and bilayer films are substantial which significantly affect elongation differences.

Table 3. Single layer and bilayer film abbreviations.

Type of Film	
Abbrev.	Meaning
Wheat gluten	Single layer film consisting of wheat gluten
Soy protein	Single layer film consisting of soy protein isolate
Corn zein	Single layer film consisting of corn zein
cs	Bilayer film containing corn zein and soy protein isolate
wc	Bilayer film containing wheat gluten and corn zein

Production Methods of Bilayer Film	
Abbrev.	Significance
pc	Corn zein poured onto a base film
ss	Soy protein isolate sprayed onto a base film
sc	Corn zein sprayed unto a base film
r	Corn zein rolled onto a base film
pw	Wheat gluten poured onto base film
ps	Soy poured onto a base film
a	Alcohol solvent and calendar rolls used to press films
h7	Calendar rolls heated to 135°C to press films
h8	Calendar rolls heated to 141°C to press films
h9	Calendar rolls heated to 146°C to press films

Bilayer Film Combinations – Visual Quality	
Abbrev.	Significance
U	Unsatisfactory combination–fragmented areas less than 49 cm^2
I	Irregular combination–uneven areas larger than 49 cm^2
F	Favorable combination–smooth film surface around 900 cm^2

X–Ray Diffraction

Testing of single layer and bilayer films showed no differences in structural formations of single layer or bilayer film types. That is, both single layer and multilayer films appear to have the same amorphous structure. Previous work by Kester and Fennema (1989a) showed that a wax–laminated film is only partially crystalline.

Summary

Unsatisfactory film combinations included pouring soy on corn, spraying or rolling wheat on any base protein material, spraying soy on wheat, or rolling soy on any base protein material. Irregular film combinations included pouring wheat on corn or soy, pouring corn on wheat or soy, pouring soy on wheat, or spraying corn on soy. An intermediate solvent with pressure and pressure plus heat both produced films that were acceptable. Other favorable film combinations included rolling corn on wheat or soy, spraying corn on wheat, and spraying soy on corn. Table 4 summarizes the advantages and limitations of forming these bilayer films.

Conclusions

Conclusions drawn from this study were:

1. Bilayer films can be constructed using two protein films.
2. The crystalline structure of bilayer films was unchanged from that of single layer films.

Table 4. Advantages and limitations of bilayer film formation techniques.

Method	Advantage	Limitation
Film formed on dried film by: 1. pouring 2. spraying 3. rolling	Thinner films Multiple film layers	Protein interactions Hydration properties pH differences
Solvent laminating	Ease of production Uniformity	Surface properties Film thickness Solvent interactions
Heat pressing	Ease of production Uniformity	Surface properties Film thickness Temperature level

3. In general, bilayer films appear to exhibit reduced tensile strength, similar oxygen permeability, enhanced percent elongation, and greatly increased water vapor permeability.
   a. Pouring wheat on corn and pouring wheat on soy improved the oxygen permeability of bilayer films beyond either single layer film.
   b. A wheat/soy combination formed with an intermediate solvent under pressure or pouring wheat on soy to form bilayer films increased the tensile strength above the same single layer films.
   c. Elongation of bilayer film was increased over single layers films by heat pressing a corn/soy, wheat/corn, or wheat/soy combinations at 135°C, heat pressing a wheat/corn or wheat/soy combinations at 141°C, heat pressing a wheat/soy combinations at 146°C, the use of an intermediate solvent and a corn/soy or wheat/corn combination under pressure, pouring wheat on soy, pouring soy on wheat, spraying corn on wheat, rolling corn on wheat, or pouring corn on wheat.
   d. Water vapor permeability of bilayer films was not improved for any bilayer formation technique.

References

ASTM. 1989. *Annual Book of ASTM Standards*. Philadelphia, Pa.: American Society for Testing and Materials.

Banker, G., A. Gore, and J. Swarbrick. 1966. Water vapour transmission properties of free polymer films. *J. Pharm. Pharmac.* 18(4): 457–466.

Brandenburg, A., C. Weller, and R. Testin. 1993. Edible films and coatings from soy protein. *J. Food Sci.* 58(5): 1086–1089.

Cheftel, J., J–L. Cuq, and D. Lorient. 1985. Amino acids, peptides and proteins. In *FoodChemistry*, ed. O. Fennema, 245–369. New York: Marcel Dekker, Inc.

Foulk, J. 1994. Formation and evaluation of bilayer protein films. M.S. thesis. Clemson University. Clemson, S.C.

Freeman Industries Inc. 1986. Specification sheets for regular grade zein. Tuckahoe, N.Y.

Gennadios, A., and C. Weller. 1990. Edible films and coatings from wheat and corn proteins. *Food Technol.* 44(10): 63–69.

Gennadios, A., and C. Weller. 1991. Edible films and coatings from soymilk and soy protein. *Cereal Foods World* 36(12): 1004–1009.

Gennadios, A., C. Weller, and C. Gooding. 1994. Measurement errors in water vapor permeability of highly permeable, hydrophilic edible films. *J. Food Eng.* 21(4): 395–409.

Hagenmaier, R., and P. Shaw. 1990. Moisture permeability of edible films made with fatty acids and (hydroxypropyl) methylcellulose. *J. Agric. Food Chem.* 38(9): 1799–1803.

Kamper, S. and O. Fennema. 1984a. Water vapor permeability of edible bilayer films. *J. Food Sci.* 49(6): 1478–1481, 1485.
Kamper, S. and O. Fennema. 1984b. Water vapor permeability of an edible, fatty acid, bilayer film. *J. Food Sci.* 49(6): 1482–1485.
Kanig, J., and H. Goodman. 1962. Evaluative procedures for film–forming materials used in pharmaceutical applications. *J. Pharm. Sci.* 51(1): 77–83.
Kester, J., and O. Fennema. 1986. Edible films and coatings: A review. *Food Technol.* 40(12): 47–59.
Kester, J., and O. Fennema. 1989a. An edible film of lipids and cellulose ethers: Barrier properties to moisture vapor transmission and structural evaluation. *J. Food Sci.* 54(6): 1383–1389.
Kester, J., and O. Fennema. 1989b. An edible film of lipid and cellulose ethers: Performance in a model frozen food system. *J. Food Sci.* 54(6): 1390–1392, 1406.
Kinsella, J. 1979. Functional properties of soy proteins. *J. Am. Oil Chem. Soc.* 56(3): 242–258.
McHugh, T., R. Avena–Bustillos, and J. Krochta. 1993. Hydrophilic edible films: Modified procedure for water vapor permeability and explanation of thickness effects. *J. Food Sci.* 58(4): 899–903.
Narayan, R. 1994. Polymeric materials from agricultural feedstocks. In *Polymers from Agricultural Co–products*, eds. M. L. Fishman, R. B. Friedman, and S. J. Huang, 2–28. Washington, D.C.: American Chemical Society.
Odian, G. 1993. *Principles of Polymerization*. New York: John Wiley & Sons Inc.
Okamoto, S. 1978. Factors affecting protein film formation. *Cereal Foods World* 23(5): 256–262.
Park, H. 1993. Personal communication. Clemson, S.C.
Park, H., and M. Chinnan. 1990. Properties of edible coatings for fruit and vegetables. ASAE Paper No. 90–6510. St. Joseph, Mich.: ASAE.
Park, H., C. Weller, P. Vergano, and R. Testin. 1992. Factors affecting barrier and mechanical properties of protein based edible, degradable films. Institute Food Technologists Paper No. 428. New Orleans, La.: Institute Food Technologists.
Park, H., J. Bunn, C. Weller, P. Vergano, and R. Testin. 1994. Water vapor permeability and mechanical properties of grain protein–based films as affected by mixtures of polyethylene glycol and glycerin plasticizers. *Transactions of the ASAE 37*(4): 1281–1285.
Pomeranz, Y. 1988. *Wheat: Chemistry and Technology*, 3rd Ed. St. Paul, Minn.: American Association of Cereal Chemists.
Rico–Pena, D., and J. Torres. 1991. Sorbic acid and potassium sorbate permeability of an edible methylcellulose–palmitic acid film: Water activity and pH effects. *J. Food Sci.* 56(2): 497–499.
Vojdani, F., and J. Torres. 1989. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose multilayer films. *J. Food Process Preserv.* 13(6): 417–430.
Vojdani, F., and J. Torres. 1990. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings: Effect of fatty acids. *J. Food Sci.* 55(3): 841–846.
Wall, J., and A. Beckwith. 1969. Relationship between structure and rheological properties of gluten proteins. *Cereal Sci. Today* 14(1): 16–18, 20–21.
Wang, J., K. Fujimoto, T. Miyazawa, and Y. Endo. 1991. Antioxidant mechanism of maize zein in power model systems against methyl linoleate: Effect of water activity and coexistence of antioxidants. *J. Agric. Food Chem*. 39(2): 351–355.
Wolf, W. 1970. Soybean proteins: Their functional, chemical and physical properties. *J. Agric. Chem.* 18(6): 969–976.